

2/3,AB/4 (Item 4 from file: 5)

11785695 Biosis No.: 199900031804

Human HtrA, an evolutionarily conserved serine protease identified as a differentially expressed gene product in osteoarthritic cartilage.

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Journal: Journal of Biological Chemistry 273 (51): p 34406-34412 Dec. 18, 1998

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Record Type: Abstract

Language: English

Abstract: The human homologue of the Escherichia coli htrA gene product was identified by the differential display analysis of transcripts expressed in osteoarthritic cartilage. This transcript was identified previously as being repressed in SV40-transformed fibroblasts (Zumbrunn, J., and Trueb, B. (1996) FEBS Lett. 398, 187-192). Levels of HtrA mRNA were elevated approx 7-fold in cartilage from individuals with osteoarthritis compared with nonarthritic controls. Differential expression of human HtrA protein was confirmed by an immunoblot analysis of cartilage extracts. Human HtrA protein expressed in heterologous systems was secreted and exhibited endoproteolytic activity, including autocatalytic cleavage. Conversion by mutagenesis of the putative active site serine 328 to alanine eliminated the enzymatic activity. Serine 328 was also found to be required for the formation of a stable complex with alpha1-antitrypsin. We have determined that the HtrA gene is highly conserved among mammalian species: the amino acid sequences encoded by HtrA cDNA clones from cow, rabbit, and guinea pig are 98% identical to human. In E. coli, a functional htrA gene product is required for cell survival after heat shock or oxidative stress; its role appears to be the degradation of denatured proteins. We propose that mammalian HtrA, with the addition of a new functionality during evolution, i.e. a mac25 homology domain, plays an important role in cell growth regulation.

1998

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2/3,AB/5 (Item 5 from file: 5)

10667979 Biosis No.: 199799289124

Degradation by proteases Lon, Clp and HtrA, of *Escherichia coli* proteins aggregated in vivo by heat shock; HtrA protease action in vivo and in vitro.

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Journal: Molecular Microbiology 22 (3): p 555-571 1996

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Record Type: Abstract

Language: English

Abstract: Thermally aggregated, endogenous proteins of *Escherichia coli* form a distinct fraction, denoted S, which is separable by sucrose-density-gradient centrifugation. It was shown earlier that DnaK, DnaJ, IbpA and IbpB heat-shock proteins are associated with the S fraction. Comparison of the rise and decay of the S fraction in mutants defective for heat-shock proteases Lon (La), Clp, HtrA (DegP, Do) and in wild-type strains made studies of proteolysis and the function of the heat-shock response possible in vivo. Different timing and the extent of action of particular proteases was revealed by the initial size and decay kinetics of the S fraction. The proteases Lon, Clp, and HtrA all participated in removal of the aggregated proteins. Mutation in the gene encoding ClpB caused the most prominent effect (47% stabilization of the S fraction). The correlation between the disappearance of the S fraction and proteolytic activity was supported by the result of the in vitro reaction. Approximately one third of the isolated S fraction was converted to trichloroacetic acid-soluble products by the purified HtrA protease. Mg-2+ ions stimulated the reaction, in contrast to the reaction of the HtrA protease with casein. The digestion of the aggregated proteins, unlike the digestion of casein, by HtrA protease in vitro was inhibited by added DnaJ, which might reflect protection of the aggregated proteins in vivo by DnaJ from excessive degradation. One might expect that such an activity of DnaJ would promote denatured protein renaturation versus proteolysis. Moreover, among the aggregated proteins that are discernible by electrophoresis, none could be identified as being more susceptible than any other to HtrA degradation. The separation pattern of these proteins before and after the in vitro digestion did not show a difference corresponding to the loss of about 30% of constituting proteins. This was interpreted as recognition by the HtrA protease of a state of protein denaturation rather than specific amino acid

sequences in particular proteins. We conclude that the fraction consisting of proteins heat-aggregated in vivo (i.e. the S fraction) contains endogenous substrates for the heat-shock proteases tested. Their use for in vitro reaction reveals information that is in some respects different from that obtained with exogenous substrates such as casein.

1996

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2/3,AB/6 (Item 6 from file: 5)

10604945 Biosis No.: 199699226090

Probing the structural role of an alpha-beta loop of maltose-binding protein by mutagenesis: Heat-shock induction by loop variants of the maltose-binding protein that form periplasmic inclusion bodies.

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Document Type: Article

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Language: English

Abstract: The maltose-binding protein (MBP) of *Escherichia coli* is the periplasmic receptor of the maltose transport system. Previous studies have identified amino acid substitutions in an alpha/beta loop of the structure of MBP that are critical for the in vivo folding. To probe genetically the structural role of this surface loop, we generated a library in which the corresponding codons 32 and 33 of malE were mutagenized. The maltose phenotype, which correlates with a biologically active structure of MBP in the periplasm, indicated a considerable variability in the loop residues compatible with a correct in vivo folding pathway of the protein. By the same genetic screens, we characterized loop-variant MBPs associated with a defective periplasmic folding pathway and aggregated into inclusion bodies. Heat-shock induction with production of misfolded loop variants was examined using both lon-lacZ and htrA-lacZ fusions. We found that the extent of formation of inclusion bodies in the periplasm of *E. coli*, from misfolded loop variant MBPs,

correlated with the level of heat-shock response regulated by the alternate heat-shock sigma factor, sigma-24.

1996

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2/3,AB/7 (Item 7 from file: 5)

09857337 Biosis No.: 199598312255

Comparison of the structure of wild-type HtrA heat shock protease and mutant HtrA proteins: A Fourier transform infrared spectroscopic study.

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Journal: Journal of Biological Chemistry 270 (19); p 11140-11146 1995

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Document Type: Article

Record Type: Abstract

Language: English

Abstract: The HtrA protease of *Escherichia coli*, identical with the DegP protease, is a 48-kDa heat shock protein, indispensable for bacterial survival only at temperatures above 42 degree C. Proteolytic activity of HtrA is inhibited by diisopropyl fluorophosphate, suggesting that HtrA is a serine protease. We have recently found that mutational alteration of serine in position 210 of the mature HtrA or of histidine in position 105 totally eliminated proteolytic activity of HtrA. However, little was known about the consequences of the mutations on HtrA conformation. In this work, Fourier transform infrared spectroscopy has been used to examine the conformation in aqueous solution of wild-type HtrA and mutant HtrAS210 and HtrAH105 proteins. The spectra were collected at different temperatures in order to gain information also on the thermal stability of the three proteins. The analysis of HtrA protein spectrum, by resolution-enhancement methods, revealed that beta-sheet is the major structural element of the conformation of HtrA. Deconvoluted as well as second derivative spectra of wild-type HtrA and mutant HtrAS210 and HtrAH105 collected at 20 degree C were identical, indicating no differences in the secondary structure of these proteins. The analysis of spectra obtained at different temperatures revealed a maximum of protein denaturation within 65-70 degree C for wild-type HtrA as well as for the HtrAS210

and HtrAH105 mutant proteins. However, the thermal denaturation pattern of wild-type HtrA revealed a lower cooperativity in the denaturation process as compared to the mutant proteins which instead behaved similarly. These data suggest that the mutations in HtrA protein induced minor changes in the tertiary structure of the protein (most likely located at the mutation sites). Our results strongly support the idea that Ser-210 and His-105 may represent two elements of the active-site triad (Ser, His, and Asp), found in most serine proteases. We have also found that in vitro, in the range from 37 to 55 degree C, the proteolytic activity of HtrA rapidly increased with temperature and that HtrA activity remained unchanged for at least 4 h at 45 degree C.

1995

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2/3,AB/8 (Item 8 from file: 5)

09579490 Biosis No.: 199598034408

Cloning, characterization and construction of htrA and htrA-like mutants of *Brucella abortus* and their survival in BALB/c mice.

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Journal: Microbial Pathogenesis 17 (1): p 23-36 1994

ISSN: 0882-4010

Document Type: Article

Record Type: Abstract

Language: English

Abstract: A genomic library of *Brucella abortus* S2308 was screened for expression of recombinant proteins recognized by sera from mice and from cattle infected with *B. abortus*. A positive clone, BA1, expressing a 50 kDa peptide was recognized by both sera. Plasmid pBA1, isolated from BA1, was shown by restriction enzyme digestion to possess a 1.9 kb insert. The nucleotide sequence of the pBA1 insert revealed an open reading frame with of 1539 bases with a coding capacity of 513 amino acids and a predicted molecular weight of 50,992. The predicted amino acid sequence showed 37% identity to *E. coli* HtrA, a temperature inducible serine protease. A second *B. abortus* htrA gene, designated htrA-like, was identified on a different cloned fragment that also encoded *B. abortus* recA. The nucleotide

sequence of the htrA-like gene revealed an open reading frame of 1422 nucleotides with a coding capacity of 474 amino acids and a predicted molecular weight of 50,155. The deduced amino acid sequence of the htrA-like gene showed 42% and 36% identity with *B. abortus* and *E. coli* HtrAs respectively. Western blotting of *E. coli* lysate containing the htrA-like gene was not recognized by sera from *B. abortus*-infected cattle or mice. *B. abortus* htrA but not htrA-like relieved the temperature sensitive phenotype and permitted growth of an *E. coli* htrA mutant at 42 degree C. *B. abortus* htrA and htrA-like mutants were constructed and their survival and growth in BALB/c mice was compared to the parental strain S2308. Splenic levels of htrA or htrA-like mutants were initially lower but after 60 days post-infection both were higher than the parental strain. Histologic analysis of hepatic inflammatory responses suggested that an initial intense granuloma formation in the htrA group was the basis for early low splenic titers of bacteria, but that failure to maintain granulomas, as did mice given the parental strain, resulted in a marked secondary rise in splenic bacterial titers.

1994

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2/3,AB/11 (Item 1 from file: 51)

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Molecular characterization of a stress-inducible gene from *Lactobacillus helveticus*.

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Journal of Bacteriology 1998 , 180 (23) 6148-6153

Language: English

Lactobacillus helveticus is widely used as a starter in the manufacture of Swiss-type cheeses and other fermented dairy products. During their manufacture *L. helveticus* is exposed to a variety of stresses such as increases in temp. and osmolarity. A stress-regulated htrA-like gene of *L. helveticus* CNRZ32 was cloned, sequenced and characterized and effects of different stresses on htrA expression analysed at the transcriptional level. The amino acid sequence of the gene exhibited 30% identity with the HtrA protein of *Escherichia coli*. Exposure of *L. helveticus* to 4% (w/v) NaCl resulted in the strongest induction in htrA expression with an 8 TIMES increase being observed. Enhanced htrA mRNA expression also occurred following exposure to heat, ethanol or puromycin. Results demonstrated that the HtrA protein facilitated growth under heat stress but had no effect under salt stress. (From En summ.)

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2/3,AB/17 (Item 1 from file: 349)

00575936

GRAM-POSITIVE BACTERIA DEPRIVED OF HtrA PROTEOLYTIC ACTIVITY AND THEIR USES
BACTERIES A GRAM POSITIF DEPOURVUES D'ACTIVITE PROTEOLYTIQUE HtrA, ET LEURS UTILISATIONS

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TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW GH GM KE LS MW SD SL SZ TZ UG
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Publication Language: French

Fulltext Word Count: 9474

English Abstract

The invention concerns bacteria strains, obtained from gram-positive bacteria whereof the genome size is not more than 3.2 Mb, and wherein the HtrA surface protease is inactive. Said strains are useful for expressing exported proteins of interest.

French Abstract

L'invention concerne des souches bactériennes, obtenues à partir de bactéries à gram-positif dont la taille du génome est au plus égale à 3,2 Mb, et dans lesquelles la protéase de surface HtrA est inactive. Ces souches sont utilisables pour l'expression de protéines d'intérêt exportées.

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2/3,AB/22 (Item 6 from file: 349)

00302514

**EXPRESSION OF HETEROLOGOUS PROTEINS IN ATTENUATED BACTERIA
USING THE HTRA-PROMOTERS**

**EXPRESSION DE PROTEINES HETEROLOGUES DANS DES BACTERIES
ATTENUEES AU MOYEN DE PROMOTEURS DU GENE HTRA**

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Publication Language: English

Fulltext Word Count: 7331

English Abstract

The invention provides a DNA construct comprising the htrA promoter sequence operably linked to a DNA sequence encoding one or more heterologous proteins, replicable expression vectors containing the constructs, and attenuated bacteria containing the constructs or vectors. The invention also provides a vaccine composition comprising an attenuated bacterium as defined above, or a fusion protein expressed from a construct as defined above, and a pharmaceutically acceptable carrier.

French Abstract

L'invention concerne un produit de recombinaison d'ADN comprenant le promoteur du htrA lie activement a une sequence d'ADN codant une ou plusieurs proteines heterologues, des vecteurs d'expression replicables contenant ledit produit de recombinaison, et des bacteries attenees contenant lesdits produit ou vecteurs. L'invention porte egalement sur une composition de vaccin comprenant une bacterie atteneue conforme a la definition ci-dessus, ou une proteine de fusion exprimee a partir d'un produit comme defini ci-dessus, et un excipient pharmaceutiquement acceptable.

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